

**Discovery of *Juniperus sabina* var. *balkanensis* R. P. Adams and A. N. Tashev
in Macedonia, Bosnia-Herzegovina, Croatia and Central and Southern Italy and relictual
polymorphisms found in nrDNA**

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ABSTRACT

Additional analyses of trnS-trnG and nrDNA from specimens from Bosnia-Herzegovina, southern and central, Italy, Croatia and Macedonia revealed the presence of *J. sabina* var. *balkanensis* in these areas west of the previously known populations in Greece, Bulgaria and western Turkey. Careful chromatogram analysis of eight (8) polymorphic sites in nrDNA revealed that nearly all of the populations of both var. *balkanensis* and var. *sabina* contained from 2 to 8 polymorphic sites. For these 8 heterozygous sites, two exclusive patterns were found in *J. sabina*. One type (GGACCCAG) was found in 16/62 plants and type 2 (ACGACAGT) was found in 4/62 plants. The majority of the plants examined (42/62) were heterozygous for 1 to 8 sites. These two nrDNA types appear to have arisen via hybridization with a *J. thurifera* ancestor. The two types appear in both v. *sabina* and v. *balkanensis* populations. Extant putative hybrids appear to have formed by crosses between present day type 1 and type 2 nrDNA. Published on-line www.phytologia.org *Phytologia* 100(2): 117-127 (Jun 22, 2018). ISSN 030319430.

KEY WORDS: *Juniperus sabina* var. *balkanensis*, *J. sabina*, distribution, nrDNA, trnS-trnG, chloroplast capture, ancient nrDNA heterozygotes.

Juniperus sabina var. *balkanensis* R. P. Adams & A. N. Tashev appears to be a product of hybrid origin between *J. sabina* L. and an ancestor of *J. thurifera* (Adams et al. 2016). Subsequent backcrossing to *J. sabina* (var. *sabina*) is hypothesized to have resulted in the capture of ancestral '*J. thurifera* like' chloroplast by *J. sabina* var. *balkanensis*. In *Juniperus*, Terry et al. (2000) suggested that chloroplast capture was involved in the distribution of cp haplotypes in *J. osteosperma* in western North America. More recently, Adams (2015a, b) found widespread hybridization and introgression between *J. maritima* and *J. scopulorum* in the Pacific northwest, with introgression from *J. maritima* into *J. scopulorum* eastward into Montana. The disparity between cpDNA and nuclear markers (nrDNA and maldehy) suggested that cp capture had occurred. In *Pinus* and other conifers, Hipkins et al. (1994) concluded that "past hybridization and associated 'chloroplast capture' can confuse the phylogenies of conifers." Bouille et al. (2011) found significant topological differences in phylogenetic trees based on cpDNA (vs. mtDNA sequences) in *Picea* that suggested organelle capture. *Juniperus sabina* L. is a smooth leaf-margined, multi-seeded juniper of the eastern hemisphere. It is very widely distributed from Spain through Europe to Kazakhstan, western China, Mongolia and Siberia (Fig. 1, Adams 2014). *Juniperus sabina* has a range that is discontinuous between Europe and central Asia; the species is generally a shrub less than 1 m tall and ranges up to 1-2 m wide. However, in the Sierra Nevada of Spain, *J. sabina* is a horizontal shrub.

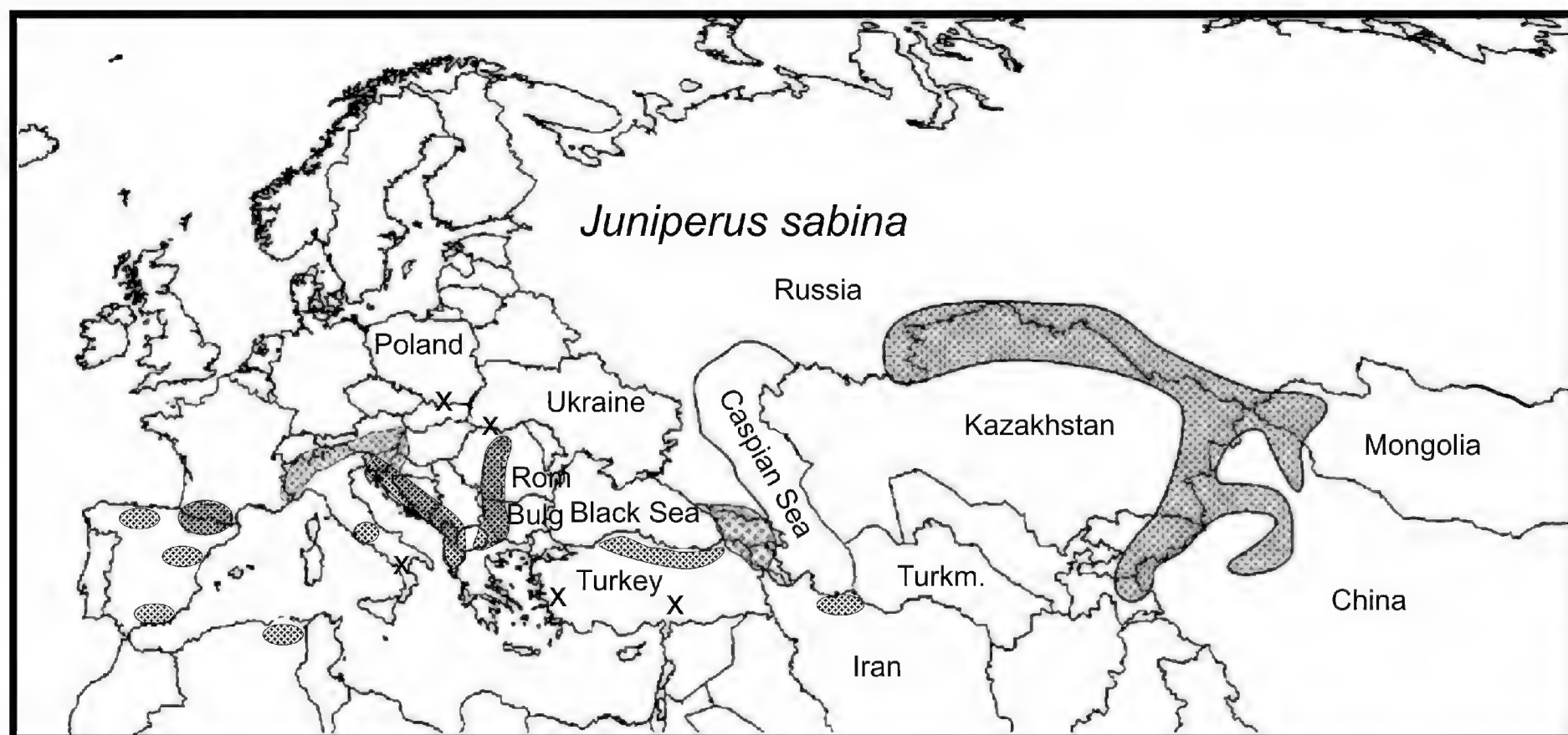


Fig. 1. Distribution (shaded areas) of *J. sabina*. x = outlying populations of *J. sabina*.

Adams et al. (2016) showed that nrDNA (ITS) did not resolve *J. sabina* populations due to the lack of sequence variation. However, their analyses (Adams et al., 2016) of cp DNA (petN-psbM, trnSG, trnDT, trnLF) revealed that *J. sabina* contained two kinds of cpDNA: typical *J. sabina* and that of *J. sabina* var. *balkanensis* cpDNA in a clade with *J. thurifera*. They recognized the taxon with the *J. thurifera* type cpDNA as a new variety: *J. s. var. balkanensis* R. P. Adams & A. N. Tashev. *Juniperus sabina* var. *balkanensis* was known only from sloping rocky limestone, at 1240 - 1630m, in the mountains of Bulgaria and northern Greece.

Subsequently, Adams et al. (2017), using samples from herbarium specimens, discovered two samples from far western Turkey that were *J. s. var. balkanensis*. However, numerous samples from

throughout Europe were confirmed to be *J. s. var. sabina* (Fig. 2). Due to the proximity of the Macedonia - Bosnia-Herzegovina - Croatia region to the populations of *J. s. var. balkanensis* in Greece, it seemed prudent to make additional collections and analyses of *J. sabina* plants from these areas to more precisely determine the distribution of *J. sabina var. balkanensis*.

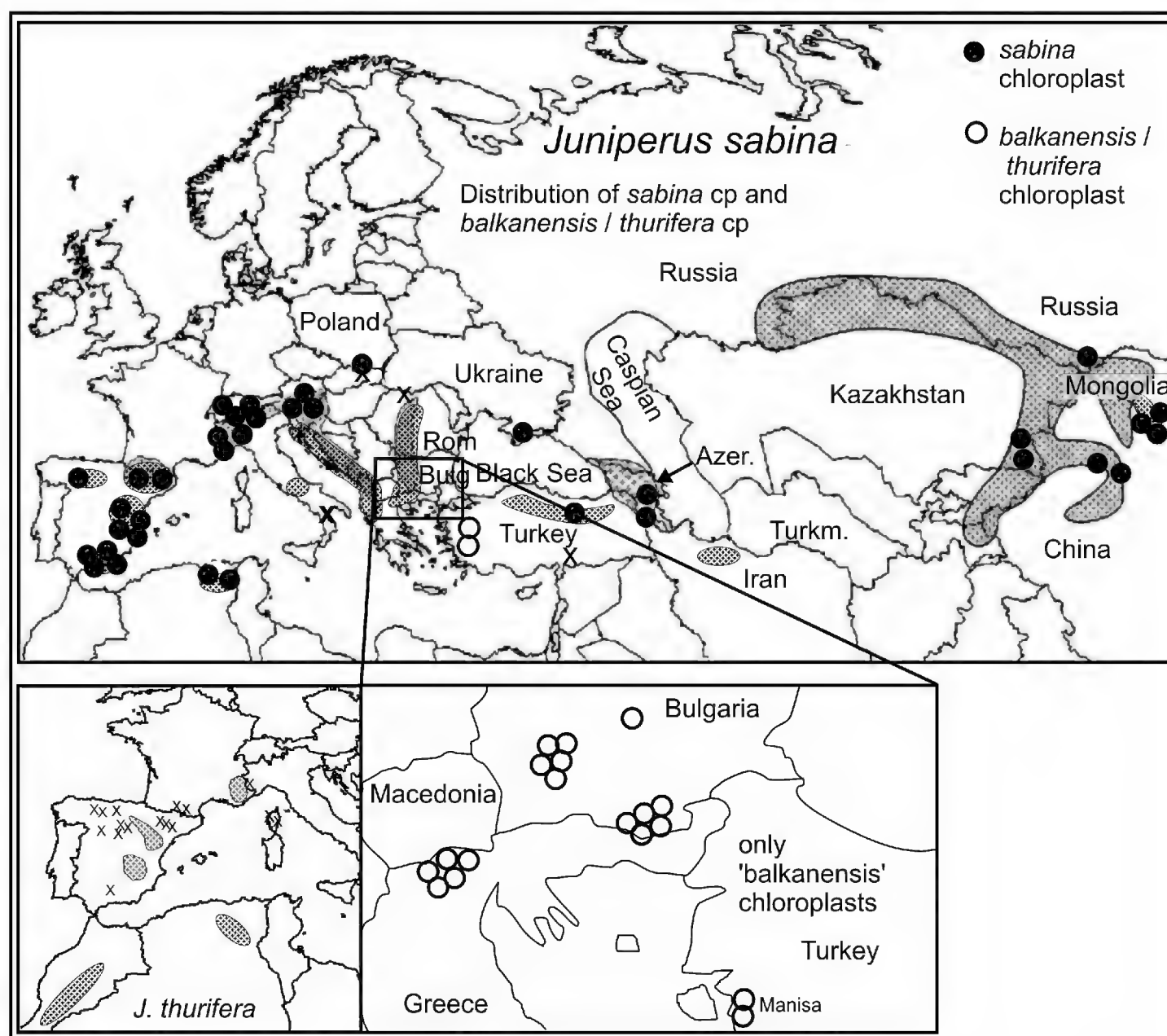


Figure 2. Distribution of *J. sabina var. balkanensis* and typical *J. sabina* chloroplast. The present day distributions of *J. thurifera* and var. *africana* (in north Africa) are shown in the insert on the lower left. (modified from Adams et al, 2017).

MATERIAL AND METHODS

Specimens used in this and previous studies: (species, popn. id., location, collection numbers): *J. chinensis*, CH, Lanzhou, Gansu, China, Adams 6765-6767; *J. sabina*: (SN), Sierra Nevada, Spain, Adams 7197, 7199, 7200; (PY), Pyrenees Mtns., Spain/ France border, Adams 7573-7577; (SW), Switzerland, Adams 7611, 7612, 7614, 7615; TS, Tian Shan Mtns., Xinjiang, China, Adams 7836-7838; Mongolia, Altai Mtns., Adams 7585-7587; Kazakhstan, Paniflor, Adams 7811-7812; Azerbaijan: Adams 14316-14320;

J. davurica (DV), 15 km se Ulan Bator, Mongolia, Adams 7252, 7253, 7601; *J. davurica var. arenaria* (AR) sand dunes, Lake Qinghai, Qinghai, China, Adams 10347-10352; river bank, Gansu, J-Q. Liu and Adams 10354-10356; *J. davurica var. mongolensis* (MS) sand dunes, 80 km sw Ulan Bator, Mongolia, Adams 7254-7256;

Collections of taxon with non-J. sabina cpDNA in Adams, Schwarzbach and Tashev (2016): (acronyms used in Fig. 7)

Bulgaria and Greece

B1-B5 Eastern Rhodopes. In protected site "Gumurdjinsky Snejnik", locality "Madzharsky Kidik". On limestone rocks above the upper border of a forest of *Fagus sylvatica* ssp. *moesiaca* with *Juniperus communis*. 41° 14' 44.7" N; 25° 15' 31.9" E. elev. 1270 m, 13 Aug. 2012, Adams 13725-13729 (A. Tashev 2012-1-5);

B6 Central Stara Planina (the Balkan). National Park "Central Balkan". Reserve "Sokolna". On a steep, rocky limestone slope, with *Sorbus aucuparia*, *S. aria*, *S. borbasii*, *Amelanchier ovalis*, *Carpinus orientalis*, *Sesleria latifolia*, *Pastinaca hirsute*, *Cephalanthera rubra*, *Laserpitium siler*, *Hieracium alpicola* etc. near a forest of *Fagus sylvatica*. 42°42'13.3" N, 25°08'10.4" E, 1501 m, 22.08.2015. Bulgaria, Adams 14721 (A. Tashev 2015 Balkan 1);

B7-B9, Ba, Bb Rila Mountain, National Park "Rila". On the eco-path, "Beli Iskar", near river Beli Iskar, in a forest with *Pinus sylvestris*, *P. peuce*, *Picea abies*, *Abies alba*, *Juniperus communis*, *J. sibirica*, *Vaccinium myrtillus*, *Rosa canina*, *Sorbus aucuparia*, *Acer hyrcanum*, *Chamaespartium sagittale*, *Hypericum perforatum*, *Thymus* sp. etc. 42°14'26.5" N, 23°32'33.8" E, 1242 m, 24.06.2015. Bulgaria, Adams 14722-14726 (A. Tashev 2015 Rila 1.1-1.3, 2.1-2.2);

G1-G5 Mt. Tsena, Greece, Adams 14727-14731 (A. Tashev 2015 So. 1-5 Tsena);

Turkey

14861 Turkey, Manisa. Spil Dağı Milli Parkı (National Park) (Tas Suret), N38.55°, E 27.42°, ca 1250 m alt., leg. A. Boratyński, K. Boratyńska, 2005, TU_05/55, KOR 44573, female

14934 Turkey, Manisa, Spil Dağı Milli Parkı (National Park), N38°, 57', E 27° 41', 1024 m., Tuğrul Mataracı 2016-1

14938 Turkey, Gümüşhane, Kürtün, Aktas village, Karakaya (Northeast Anatolia), 40° 36' 03" N, 38° 53' 21" E., 2376 m. Coll. A. Kandemir 10745.

Samples new for this study: (with Lab Acc. ID = Adams xxxxx)

Bosnia-Herzegovian

Juniperus sabina var. *balkanensis*, on calcareous, dolomite, between Mt. Cvrstica and Mt. Cabulja, 43° 34' 18.09" N, 17° 30' 39.88" E., 1460m, 7 June 2017, Bosnia-Herzegovina, Coll. F. Bogunic & Sonja Siljak-Yakovlev, Lab Acc. Robert P. Adams 15277-15281.

Croatia

Juniperus sabina var. *balkanensis*, Mt. Biokovo, Vosac, Strbina, 1200 m, NB! 4n. 43° 18' 34.9" N, 17° 02' 36.3" E., 1300m, 15 June 2017, Croatia, Coll. Sonja Siljak-Yakovlev, Lab Acc. Robert P. Adams 15282-15286.

Juniperus sabina var. *balkanensis*, 44°32'36" N, 15°10'09" E, 1080 m, fall 2017, Country: Velebit, Croatia, Coll. Katarzyna Marcysiak, Lab Acc. Robert P. Adams 15343-15346.

Macedonia

Juniperus sabina var. *balkanensis*, 41° 39' 18.16" N, 20° 44' 01.21" E., 4519 ft, Oct 2017, Macedonia, Mavrovo area. Coll. Katarzyna Marcysiak, Lab Acc. Robert P. Adams 15311-15315.

Juniperus sabina var. *balkanensis*, 41° 35' 46.61" N, 20° 39' 04.94" E., 4755 ft, Oct 2017, Macedonia, Gaichnik area, Coll. Katarzyna Marcysiak, Lab Acc. Robert P. Adams 15316-15320.

Italy

Juniperus sabina var. *balkanensis*, P.N. del Pollino (San Lorenzo Bellizzi, CS), Timpa di San Lorenzo, su roccia calcarea, leaves from herbarium specimen [in Pisa, (PI)], 39.91349° N, 16.28578° E, 1436 m, 18 Aug 2017, Calabria, Italy, Coll. Francesco Roma-Marzio et L. Peruzzi, Lab Acc. Robert P. Adams 15365

Juniperus sabina var. *balkanensis*. Valle di Selva Romana (Pennapiedimonte, Chieti), pendii rupestri, 1600-1900 m, 42° 07' 41" N, 14° 07' 18" W., 5411 ft, 01 Aug 2002, Abruzzo, Italy, Coll. F. Conti (APP No. 1701), ex Fabrizio Bartolucci, received 14 Apr 2018, Lab Acc. Robert P. Adams 15413

Juniperus sabina var. *balkanensis*. Vallone di Pennapiedimonte (Pennapiedimonte, Chieti), pascoli, 800-900 m, 42° 09' 01" N, 14° 11' 02" W., 2017 ft, 05 May 1999, Abruzzo, Italy, Coll. F. Conti (APP No. 13142), ex Fabrizio Bartolucci, received 14 Apr 2018, Lab Acc. Robert P. Adams 15414

Juniperus sabina var. *balkanensis*. Gran Sasso - M.te Camicia, loc. il Gravone (Castelli, Teramo), pendii rupestri, 1700 m, 42° 26' 45" N, 13° 44' 06" W., 5484 ft, 02 Nov 1996, Abruzzo, Italy, Coll. F. Conti (APP No. 17471), ex Fabrizio Bartolucci, received 14 Apr 2018, Lab Acc. *Robert P. Adams 15415*

Juniperus sabina var. *balkanensis*. M. S. Domenico (Pizzoferrato, Chieti), 41° 55' 28" N, 14° 14' 12" W., 3967 ft, 17 June 1995, Abruzzo, Italy, Coll. F. Conti (APP No. 33835), ex Fabrizio Bartolucci, received 14 Apr 2018, Lab Acc. *Robert P. Adams 15416*

Juniperus sabina var. *balkanensis*. Scatafosse (Villavallelonga, L'Aquila), 41° 47' 55" N, 13° 41' 13" W., 5809 ft, 01 Aug 2001, Abruzzo, Italy, Coll. F. Conti (APP No. 50172), ex Fabrizio Bartolucci, received 14 Apr 2018, Lab Acc. *Robert P. Adams 15417*

Voucher specimens for all collections are deposited at Baylor University Herbarium (BAYLU), Herbarium (University of Forestry, Sofia, Bulgaria) and Herbarium Apenninicum (APP).

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit (Qiagen, Valencia, CA) as per manufacturer's instructions. Amplifications were performed in 30 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 µl 2x buffer E (petN, trnD-T, trnL-F, trnS-G) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 µM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM MgCl₂ according to the buffer used) 1.8 µM each primer. See Adams, Bartel and Price (2009) for the ITS and petN-psbM primers utilized. The primers for trnD-trnT, trnL-trnF and trnS-trnG regions have been previously reported (Adams and Kauffmann, 2010). The PCR reaction was subjected to purification by agarose gel electrophoresis. In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit (Qiagen, Valencia, CA). The gel purified DNA band with the appropriate sequencing primer was sent to McLab Inc. (San Francisco) for sequencing. 2.31 (Technelysium Pty Ltd.).

RESULTS

All the plants sampled in Macedonia, Bosnia-Herzegovina, Croatia and Calabria and Abruzzo areas of Italy were *J. s.* var. *balkanensis* based on cp DNA (Table 1). The revised distribution of *v. balkanensis* is shown in Figure 3. All known locations of *v. balkanensis* are in a relatively small geographical area.

Before discussing the nrDNA patterns, it should be noted that there are recent papers analyzing the inheritance of nrDNA in the Cupressaceae. Adams and Matsumoto (2016) analyzed 3 variable sites of nrDNA from synthetic crosses between *Cryptomeria japonica* cv. Haara and cv. Kumotooshi (= cv. Haara x Kumotooshi, ie., a backcross). They found that 3 of the 7 progeny had nrDNA very similar to that of the Haara x Kumo parent. In contrast, 4 of the 7 progeny had nrDNA exactly like the Haara parent. This appears to suggest that nrDNA polymorphisms can revert to that of a recurrent parent in the case of backcrossing. Adams, Miller and Low (2016) examined 8 variable nrDNA sites in the parents (*Hesperocyparis arizonica*, *H. macrocarpa*), and their 18 artificial hybrid progeny. Each of the 18 hybrids were heterozygous for all 8 nrDNA sites. This study is very relevant to the present study, because *Hesperocyparis* (= *Cupressus* in the western hemisphere) is very closely related to *Juniperus* (Little et al. 2004, Terry, et al. 2012, Terry and Adams, 2015, Terry et al. 2016) and because there are no verified artificial hybrids of *Juniperus* available to the authors for the examination of the inheritance of

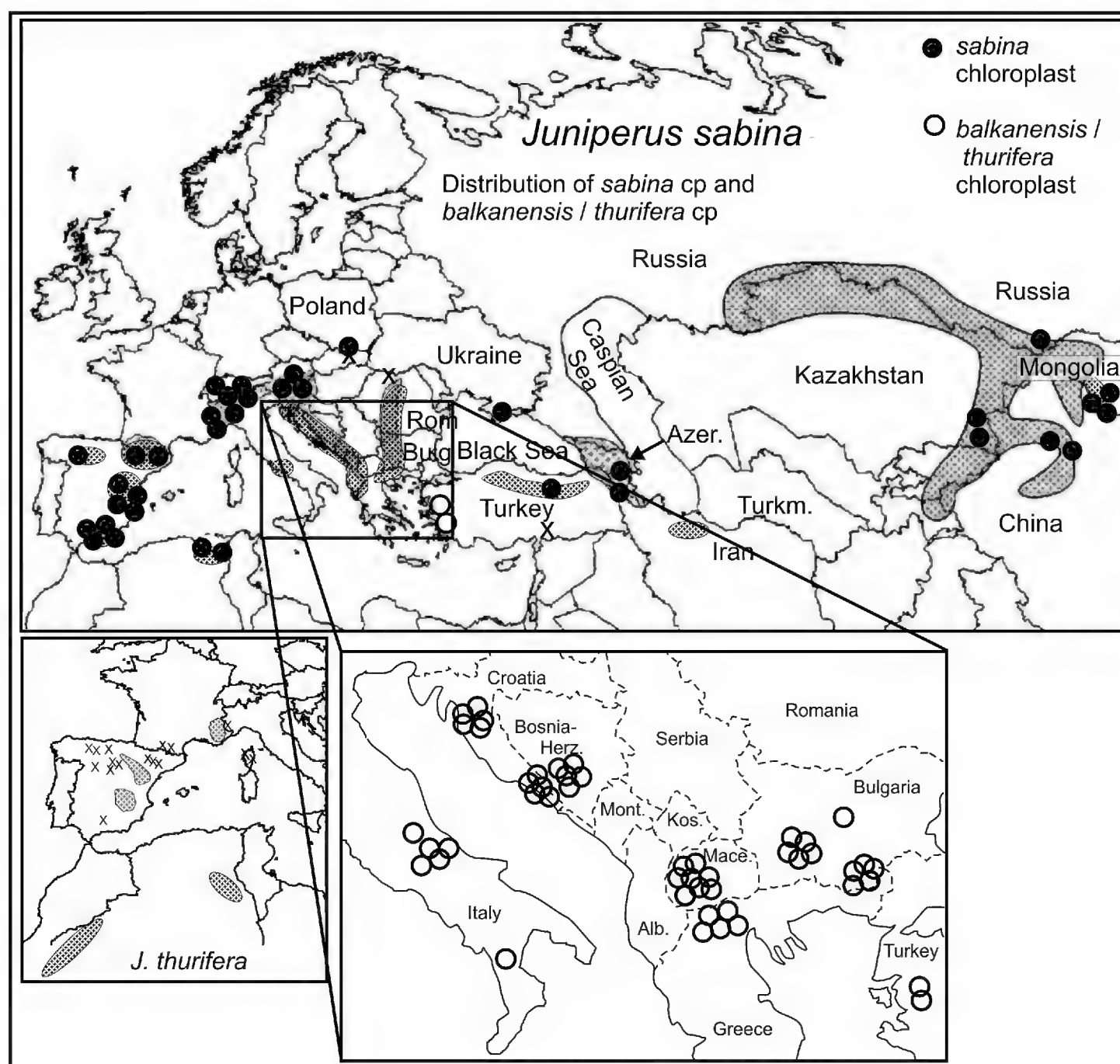


Figure 3. Revised distribution of *J. sabina* var. *sabina* (dark circles) and *J. s.* var. *balkanensis* (open circles) based on cp DNA (present study and from Adams et al. 2017)

nrDNA in *Juniperus*, the Adams, Miller and Low (2016) research on *Hesperocyparis* stands as a proxy for the inheritance of nrDNA in *Juniperus* and thus, their study in *Hesperocyparis* is surely applicable to *Juniperus*. So, we can confidently assume that nrDNA is inherited by complementation as found in *Hesperocyparis*, *Cryptomeria* and all other conifers. It should be noted that Adams, Miller and Low (2016) sequenced cp markers and confirmed that the cp genome is inherited via pollen (paternally inherited) in *Hesperocyparis* (and presumably *Juniperus*).

Aligning *J. sabina* and *J. thurifera* nrDNA sequences revealed that the taxa differ by SNPs at 22 sites, all in ITS1 or ITS2. However, a close examination of the nrDNA sequencing chromatograms revealed that only 8 of the 22 sites contained heterozygous peaks. The 8 sites were (with position): 352(R), 391(S), 432(R), 606(M), 785(Y), 999(M), 1046(R), 1047(K). Two nrDNA types were found considering these 8 sites. One nrDNA type (GGACCCAG) was found in 16/62 plants and type 2 (ACGACAGT) was found in 4/62 plants. The majority of the plants examined (42/62) were heterozygous for 1 to 8 sites (Table 1). Note that 33/42 heterozygous individuals were heterozygous for all 8 sites. The frequency of heterozygous sites was: 1,1,1,3,2,1,0,33 (for plants containing from 1 to 8 polymorphic sites, respectively). Heterozygous plants are somewhat randomly distributed (Fig. 4). It is interesting that 4/4 Spain and 2/3 Switzerland plants were homozygous for the 8 sites. Only 2/7 plants from the far East were homozygous. Plants of var. *balkanensis* seem to be a bit more heterozygous (only 12/48 were homozygous, Fig. 4).

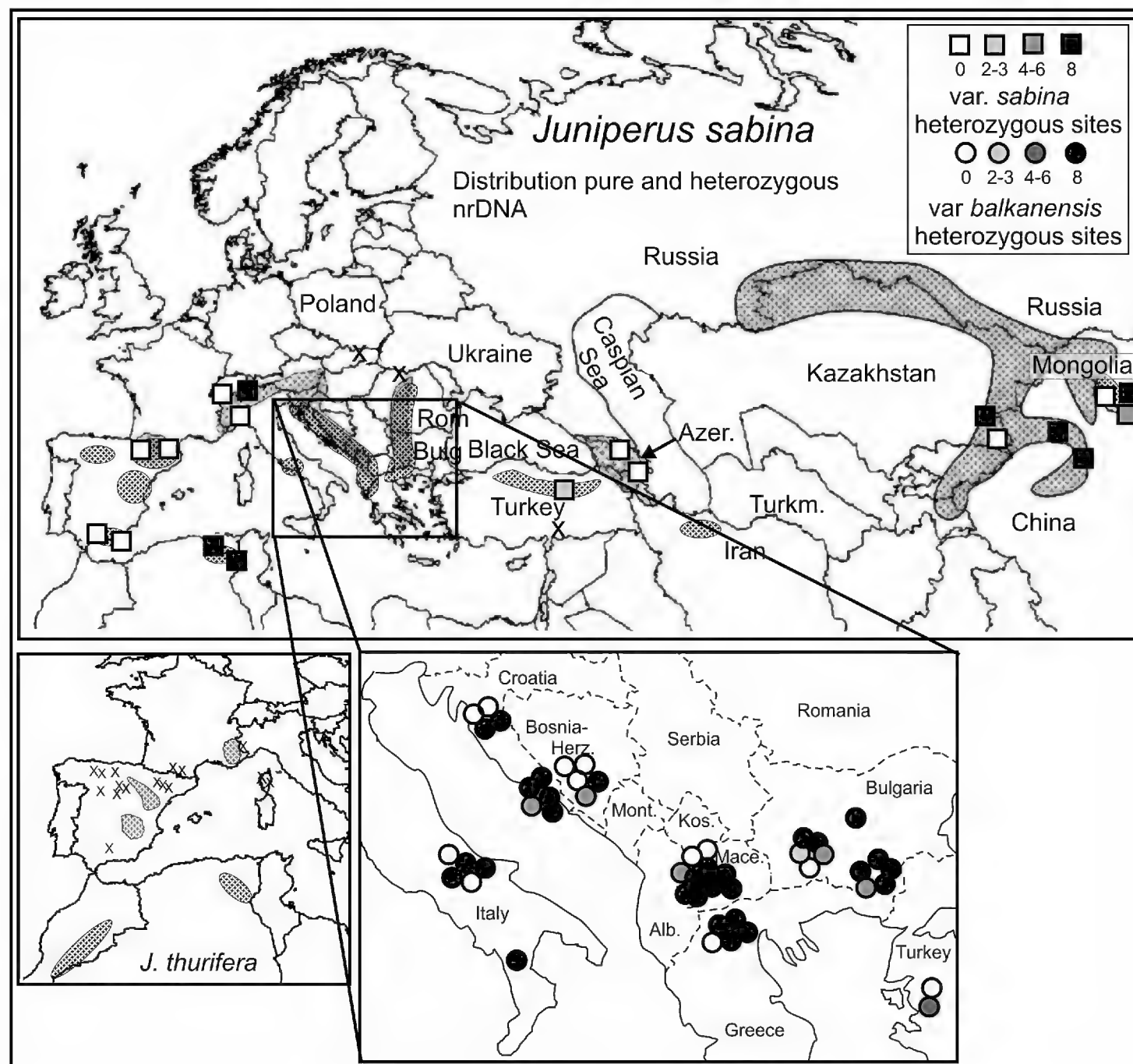


Figure 4. Distribution of homozygosity and heterozygosity among the 8 nrDNA polymorphic sites.

A more detailed examination of nrDNA type 1 (16/20) and type 2 (4/20) homozygous plants (Table 2) shows that both type 1 and type 2 plants were present in the Switzerland samples. One type 2 plant was found in Rila Mtn., Bulgaria and 2 were from Azerbaijan. All 16 type 2 nrDNAs share 4 bp sites with *J. thurifera*, and the four type 2 plants share 3 bp sites with *J. thurifera* (Table 2). It is strange that type 1 nrDNA contains 4 bp, typical of *J. thurifera* (in the 8 sites), and type 2 contain 3 different bp typical of *J. thurifera* (below and Table 2), but these are mutually exclusive in types 1 and 2.

most common sabina pattern (type 1)	G G A C C C A G	4 sites in common with <i>J. thurifera</i>
7083, thurifera, France, Morocco²	A G G C T C G G	
2nd common sabina pattern (type 2)	A C G A C A G T	3 sites in common with <i>J. thurifera</i>

Crossing type 1 (GGACCCAG) x type 2 (ACGACAGT) gives RSRMCMRK (see below), which differs at only site 5, from the putative hybrids (below and Table 1) of RSRMYMRK. Our data indicates that crossing between types 1 and 2 nrDNA types seem common, 42/62 plants were RSRMYMRK, however, none were RSRMCMRK! (the product of type 1 x type 2). These two nrDNA types may have arisen via hybridization with a *J. thurifera* ancestor and subsequent backcrossing to *J. sabina*.

most common sabina pattern (type 1)	G G A C C C A G
2nd common sabina pattern (type 2)	A C G A C A G T
cross between <i>J. sabina</i> type1 x type2	R S R M C M R K
Putative 'hybrid' pattern (table 1)	R S R M Y M R K

At our present level of understanding, the distributions of *J. s. var. balkanensis* and *J. thurifera* do not appear to overlap, negating modern hybridization. However, there were large changes in plant distributions in the Pleistocene and earlier, it seem probable that *J. thurifera*-like ancestors were sympatric with *J. sabina*, and presenting opportunities for chloroplast capture from *J. thurifera*.

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LITERATURE CITED

- Adams, R. P. 2014. The junipers of the world: The genus *Juniperus*. 4th ed. Trafford Publ., Victoria, BC.
- Adams, R. P. 2015a. Allopatric hybridization and introgression between *Juniperus maritima* R. P. Adams and *J. scopulorum* Sarg.: Evidence from nuclear and cpDNA and leaf terpenoids. *Phytologia* 97: 55-66.
- Adams, R. P. 2015b. Allopatric hybridization and introgression between *Juniperus maritima* R. P. Adams and *J. scopulorum* Sarg. II. Additional Evidence from nuclear and cpDNA genes in Montana, Wyoming, Idaho and Utah. *Phytologia* 97: 189-199.
- Adams, R. P., J. A. Bartel and R. A. Price. 2009. A new genus, *Hesperocypris*, for the cypresses of the new world. *Phytologia* 91: 160-185.
- Adams, R. P. and M. E. Kauffmann. 2010. Geographic variation in nrDNA and cp DNA of *Juniperus californica*, *J. grandis*, *J. occidentalis* and *J. osteosperma* (Cupressaceae). *Phytologia* 92: 266-276.
- Adams, R. P. and Asako Matsumoto. 2016. Inheritance of nrDNA in artificial hybrids of *Cryptomeria japonica* cv. *Haara* and *C. japonica* cv. *Kumotoshi*. *Phytologia* 98: 37-41.
- Adams, R. P., M. Miller and C. Low. 2016. Inheritance of nrDNA in artificial hybrids of *Hesperocypris arizonica* x *H. macrocarpa*. *Phytologia* 98: 277-283.
- Adams, R., A. E. Schwarzbach and A. N. Tashev. 2016a. Chloroplast capture in *Juniperus sabina* var. *balkanensis* R. P. Adams and A. N. Tashev, from the Balkan peninsula: A new variety with a history of hybridization with *J. thurifera*. *Phytologia* 98: 100-111.
- Adams, R. P., A. Boratynski, T. Mataraci, A. N. Tashev and A. E. Schwarzbach. 2017. Discovery of *Juniperus sabina* var. *balkanensis* R. P. Adams and A. N. Tashev in southwestern Turkey. *Phytologia* 99: 22-31.
- Bouille, M., S. Senneville and J. Bousquet. 2011. Discordant mtDNA and cpDNA phylogenies indicate geographic speciation and reticulation as driving factors for the diversification of the genus *Picea*. *Tree Genetics & Genomes* 7: 469-484.
- Hipkins, V. D., K. V. Krutovskii and S. H. Strauss. 1994. Organelle genomes in conifers: structure, evolution, and diversity. *Forest Genetics* 1: 179-189.
- Little, D. P., A. E. Schwarzbach, R. P. Adams and C-F. Hsieh. 2004. The circumscription and phylogenetic relationships of *Callitropsis* and the newly described genus *Xanthocypris* (Cupressaceae). *Am. J. Bot.* 91: 1872-1881.
- Terry, R. G., R. S. Nowak and R. J. Tausch. 2000. Genetic variation in chloroplast and nuclear ribosomal DNA in Utah juniper (*Juniperus osteosperma*, Cupressaceae): evidence of interspecific gene flow. *Am. J. Bot.* 87: 250-258.

- Terry, R. G., J. A. Bartel and R. P. Adams. 2012. Phylogenetic relationships among the new world cypresses (*Hesperocyparis*; Cupressaceae): evidence from noncoding chloroplast DNA sequences. *Plant Syst. Evol.* DOI 10.1007/s00606-012-0696-3.
- Terry, R. G. and R. P. Adams. 2015. A molecular re-examination of phylogenetic relationships among *Juniperus*, *Cupressus*, and the *Hesperocyparis-Callitropsis-Xanthocyparis* clades of Cupressaceae. *Phytologia* 97: 67-75.
- Terry, R. G., M. I. Pyne, J. A. Bartel and R. P. Adams. 2016. A molecular biogeography of the New World cypresses (*Callitropsis*, *Hesperocyparis*; Cupressaceae). *Plant Syst. Evol.* DOI 10.1007/s00606-016-1308-4.

Table 1. Survey of *J. sabina* and classification based on ITS and trnS-trnG sequences. Putative hybrids and backcrosses [*J. sabina* x ancestor of *J. thurifera*] based on ITS polymorphisms are in **bold**.

coll. #, location	trnS-trnG classification (ie. cp genome)	ITS classif.	polymorphic sites [†]								ITS #poly/8 sites
			1	2	3	4	5	6	7	8	
14861 Spil Dađi, Turk., Boratynski	v. balkanensis	sabina	R	G	R	C	Y	C	R	G	4
14934 Spil Dagi, Turkey, Mataraci	v. balkanensis	sabina	G	G	A	C	C	C	A	G	0
13725 eastern Rhodopes, Bulgaria	v. balkanensis	sabina	G	G	A	M	Y	M	R	K	5
13726 eastern Rhodopes, Bulgaria	v. balkanensis	sabina	R	S	R	M	Y	M	R	K	8
13727 eastern Rhodopes, Bulgaria	v. balkanensis	sabina	R	S	R	M	Y	M	R	K	8
13728 eastern Rhodopes, Bulgaria	v. balkanensis	sabina	R	S	R	M	Y	M	R	K	8
13729 eastern Rhodopes, Bulgaria	v. balkanensis	sabina	R	S	R	M	Y	M	R	K	8
14721 Sokolna reserve, Bulgaria	v. balkanensis	sabina	R	S	R	M	Y	M	R	K	8
14722 Rila Mtn., Bulgaria	v. balkanensis	sabina	G	G	A	C	Y	C	A	G	1
14723 Rila Mtn., Bulgaria	v. balkanensis	sabina	A	C	G	A	T	A	G	T	0
14724 Rila Mtn., Bulgaria	v. balkanensis	sabina	R	G	A	C	Y	M	A	G	3
14725 Rila Mtn., Bulgaria	v. balkanensis	sabina	R	S	R	M	Y	M	R	K	8
14726 Rila Mtn., Bulgaria	v. balkanensis	sabina	R	S	R	M	Y	M	R	K	8
14727 Tsena Mtn., Greece	v. balkanensis	sabina	R	S	R	M	Y	M	R	K	8
14728 Tsena Mtn., Greece	v. balkanensis	sabina	G	G	A	C	C	C	A	G	0
14729 Tsena Mtn., Greece	v. balkanensis	sabina	R	S	R	M	Y	M	R	K	8
14730 Tsena Mtn., Greece	v. balkanensis	sabina	R	S	R	M	Y	M	R	K	8
14731 Tsena Mtn., Greece	v. balkanensis	sabina	R	S	R	M	Y	M	R	K	8
15311 Mavrovo, Macedonia	v. balkanensis	sabina	R	S	R	M	Y	M	R	K	8
15312 Mavrovo, Macedonia	v. balkanensis	sabina	R	S	R	M	Y	M	R	K	8
15313 Mavrovo, Macedonia	v. balkanensis	sabina	G	G	A	C	C	C	A	G	0
15314 Mavrovo, Macedonia	v. balkanensis	sabina	R	S	R	M	Y	M	R	K	8
15315 Mavrovo, Macedonia	v. balkanensis	sabina	R	S	R	M	Y	M	R	K	8
15316 Gaichnik, Macedonia	v. balkanensis	sabina	R	S	R	M	Y	M	R	K	8
15317 Gaichnik, Macedonia	v. balkanensis	sabina	R	S	R	M	Y	M	R	K	8
15318 Gaichnik, Macedonia	v. balkanensis	sabina	R	S	R	M	Y	M	R	K	8
15319 Gaichnik, Macedonia	v. balkanensis	sabina	G	G	A	C	C	C	A	G	0
15320 Gaichnik, Macedonia	v. balkanensis	sabina	R	C	R	A	T	M	R	K	5
15277 Mt. Cabulja, Bosnia-Herze.	v. balkanensis	sabina	R	S	R	A	Y	M	R	K	6
15278 Mt. Cvsnica, Bosnia-Herze.	v. balkanensis	sabina	R	S	R	M	Y	M	R	K	8
15279 Mt. Cabulja, Bosnia-Herze.	v. balkanensis	sabina	G	G	A	C	C	C	A	G	0
15280 Mt. Cabulja, Bosnia-Herze.	v. balkanensis	sabina	G	G	A	C	C	C	A	G	0
15281 Mt. Cabulja, Bosnia-Herze.	v. balkanensis	sabina	G	G	A	C	C	C	A	G	0
15282 Mt. Biokovo, Croatia	v. balkanensis	sabina	R	S	R	M	Y	M	R	K	8
15283 Mt. Biokovo, Croatia	v. balkanensis	sabina	G	G	A	C	Y	M	R	K	4
15284 Mt. Biokovo, Croatia	v. balkanensis	sabina	R	S	R	M	Y	M	R	K	8
15285 Mt. Biokovo, Croatia	v. balkanensis	sabina	R	S	R	M	Y	M	R	K	8
15286 Mt. Biokovo, Croatia	v. balkanensis	sabina	R	S	R	M	Y	M	R	K	8
15343 Velebit, Croatia	v. balkanensis	sabina	G	G	A	C	C	C	A	G	0
15344 Velebit, Croatia	v. balkanensis	sabina	G	G	A	C	C	C	A	G	0
15345 Velebit, Croatia	v. balkanensis	sabina	R	S	R	M	Y	M	R	K	8
15346 Velebit, Croatia	v. balkanensis	sabina	R	S	R	M	Y	M	R	K	8

15365 Calabria area, southern Italy	v. <i>balkanensis</i>	<i>sabina</i>	R S R M Y M R K	8
15413 Abruzzo area, central Italy	v. <i>balkanensis</i>	<i>sabina</i>	R S R M Y M R K	8
15414 Abruzzo area, central Italy	v. <i>balkanensis</i>	<i>sabina</i>	R S R M Y M R K	8
15415 Abruzzo area, central Italy	v. <i>balkanensis</i>	<i>sabina</i>	G G A C C C A G	0
15416 Abruzzo area, central Italy	v. <i>balkanensis</i>	<i>sabina</i>	G G A C C C A G	0
15414 Abruzzo area, central Italy	v. <i>balkanensis</i>	<i>sabina</i>	R S R M Y M R K	8
13167 Algeria	v. <i>sabina</i>	<i>sabina</i>	R S R M Y M R K	8
13168 Algeria	v. <i>sabina</i>	<i>sabina</i>	R S R M Y M R K	8
7197 Sierra Nevada, Granada, Spain	v. <i>sabina</i>	<i>sabina</i>	G G A C C C A G	0
7199 Sierra Nevada, Granada, Spain	v. <i>sabina</i>	<i>sabina</i>	G G A C C C A G	0
7573 Sallent deGallego, Spain	v. <i>sabina</i>	<i>sabina</i>	G G A C C C A G	0
7574 Sallent deGallego, Spain	v. <i>sabina</i>	<i>sabina</i>	G G A C C C A G	0
7611 Switzerland	v. <i>sabina</i>	<i>sabina</i>	R S R M Y M R K	8
7612 Switzerland	v. <i>sabina</i>	<i>sabina</i>	G C A C C C A G	0
7614 Switzerland	v. <i>sabina</i>	<i>sabina</i>	A C G A T A G T	0
14938 northeast Turkey Kandemir	v. <i>sabina</i>	<i>sabina</i>	R G R C T A G T	2
14316 Azerbaijan	v. <i>sabina</i>	<i>sabina</i>	A C G A C A G T	0
14317 Azerbaijan	v. <i>sabina</i>	<i>sabina</i>	A C G A C A G T	0
7811 Kazakhstan, Paniflor	v. <i>sabina</i>	<i>sabina</i>	R S R M Y M R K	8
7812 Kazakhstan, Paniflor	v. <i>sabina</i>	<i>sabina</i>	G G A C C C A G	0
7585 Mongolia, Altair Mtns.	v. <i>sabina</i>	<i>sabina</i>	R S R M Y M R K	8
7586 Mongolia, Altair Mtns	v. <i>sabina</i>	<i>sabina</i>	A C G A Y M R K	4
7587 Mongolia, Altair Mtns	v. <i>sabina</i>	<i>sabina</i>	G G A C C C A G	0
7836 China, Heaven Lake, Xinjiang	v. <i>sabina</i>	<i>sabina</i>	R S R M Y M R K	8
7837 China, Heaven Lake, Xinjiang	v. <i>sabina</i>	<i>sabina</i>	R S R M Y M R K	8
most common <i>sabina</i> pattern			G G A C C C A G	0
7083, <i>thurifera</i> , France ²			A G G C T C G G	0
9420, <i>thurifera</i> v. <i>africana</i> , Morocco ²			A G G C T C G G	0
2nd most common <i>sabina</i> pattern			A C G A C A G T	0

¹Eight polymorphic sites (1-8): R352, S391, R432, M606, Y785, M999, R1046, K1047.

²This pattern, A G G C T C G G, was also found in all *J. thurifera* samples examined, to date (14 *J. thurifera* samples from Corse, Morocco, France and Spain, Adams, unpublished)

Table 2. *Juniperus sabina* classified based on ITS homozygous for all 8 polymorphic sites. Putative hybrids (heterozygous for the 8 polymorphic sites) were excluded.

coll. #, location	trnS-trnG classification	ITS classif.	nrDNA type	polymorphic sites ¹								# sites in common with <i>J. thurifera</i>
				1	2	3	4	5	6	7	8	
14934 Spil Dagi, Turkey, Mataraci	<i>v. balkanensis</i>	<i>sabina</i>	1	G	G	A	C	C	C	A	G	4
14728 Tsena Mtn., Greece	<i>v. balkanensis</i>	<i>sabina</i>	1	G	G	A	C	C	C	A	G	4
15313 Mavrovo, Macedonia	<i>v. balkanensis</i>	<i>sabina</i>	1	G	G	A	C	C	C	A	G	4
15319 Gaichnik, Macedonia	<i>v. balkanensis</i>	<i>sabina</i>	1	G	G	A	C	C	C	A	G	4
15279 Mt. Cabulja, Bosnia-Herze.	<i>v. balkanensis</i>	<i>sabina</i>	1	G	G	A	C	C	C	A	G	4
15280 Mt. Cabulja, Bosnia-Herze.	<i>v. balkanensis</i>	<i>sabina</i>	1	G	G	A	C	C	C	A	G	4
15281 Mt. Cabulja, Bosnia-Herze.	<i>v. balkanensis</i>	<i>sabina</i>	1	G	G	A	C	C	C	A	G	4
15343 Velebit, Croatia	<i>v. balkanensis</i>	<i>sabina</i>	1	G	G	A	C	C	C	A	G	4
15344 Velebit, Croatia	<i>v. balkanensis</i>	<i>sabina</i>	1	G	G	A	C	C	C	A	G	4
7197 Sierra Nevada, Granada, Spain	<i>v. sabina</i>	<i>sabina</i>	1	G	G	A	C	C	C	A	G	4
7199 Sierra Nevada, Granada, Spain	<i>v. sabina</i>	<i>sabina</i>	1	G	G	A	C	C	C	A	G	4
7573 Sallent deGallego, Spain	<i>v. sabina</i>	<i>sabina</i>	1	G	G	A	C	C	C	A	G	4
7574 Sallent deGallego, Spain	<i>v. sabina</i>	<i>sabina</i>	1	G	G	A	C	C	C	A	G	4
7812 Kazakhstan, Paniflor	<i>v. sabina</i>	<i>sabina</i>	1	G	G	A	C	C	C	A	G	4
7587 Mongolia, Altair Mtns	<i>v. sabina</i>	<i>sabina</i>	1	G	G	A	C	C	C	A	G	4, Note Type 1!
7614 Switzerland	<i>v. sabina</i>	<i>sabina</i>	2	A	C	G	A	T	A	G	T	3, Note Type 2!
14723 Rila Mtn., Bulgaria	<i>v. balkanensis</i>	<i>sabina</i>	2	A	C	G	A	T	A	G	T	3
14316 Azerbaijan	<i>v. sabina</i>	<i>sabina</i>	2	A	C	G	A	C	A	G	T	3
14317 Azerbaijan	<i>v. sabina</i>	<i>sabina</i>	2	A	C	G	A	C	A	G	T	3
7612 Switzerland	<i>v. sabina</i>	<i>sabina</i>	1	G	C	A	C	C	C	A	G	3
				bases in common (bold) w <i>thurifera</i>								
most common sabina pattern(type 1)			1	G	G	A	C	C	C	A	G	4 sites in common
7083, thurifera, France, Morocco²				A	G	G	C	T	C	G	G	8
2nd common sabina pattern(type 2)			2	A	C	G	A	C	A	G	T	3 sites in common
14861 Spil Dagi, Turk., Boratynski	<i>v. balkanensis</i>	<i>sabina</i>		R	G	R	C	Y	C	R	G	4
13725 eastern Rhodopes, Bulgaria	<i>v. balkanensis</i>	<i>sabina</i>		G	G	A	M	Y	M	R	K	5
14722 Rila Mtn., Bulgaria	<i>v. balkanensis</i>	<i>sabina</i>		G	G	A	C	Y	C	A	G	1
14724 Rila Mtn., Bulgaria	<i>v. balkanensis</i>	<i>sabina</i>		R	G	A	C	Y	M	A	G	3
15320 Gaichnik, Macedonia	<i>v. balkanensis</i>	<i>sabina</i>		R	C	R	A	T	M	R	K	5
15277 Mt. Cabulja, Bosnia-Herze.	<i>v. balkanensis</i>	<i>sabina</i>		R	S	R	A	Y	M	R	K	6
15283 Mt. Biokovo, Croatia	<i>v. balkanensis</i>	<i>sabina</i>		G	G	A	C	Y	M	R	K	4
14938 northeast Turkey Kandemir	<i>v. sabina</i>	<i>sabina</i>		R	G	R	C	T	A	G	T	2
7586 Mongolia, Altair Mtns	<i>v. sabina</i>	<i>sabina</i>		A	C	G	A	Y	M	R	K	4
most common sabina pattern(type 1)			1	G	G	A	C	C	C	A	G	
2nd common sabina pattern(type 2)			2	A	C	G	A	C	A	G	T	
cross between <i>J. sabina</i> type1 x type2			1x2	R	S	R	M	C	M	R	K	
Putative 'hybrid' pattern (table 1)				R	S	R	M	Y	M	R	K	

¹Eight polymorphic sites (1-8): R352, S391, R432, M606, Y785, M999, R1046, K1047.²This pattern, AGGCTCGG, was found in all *J. thurifera* samples examined, to date (14 *J. thurifera* samples from Corse, Morocco, France and Spain, Adams, unpublished)